

## THE MICROBIOLOGY OF CONVERSION OF LONG-CHAIN FATTY ACIDS (LCFA) TO BIOGAS

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### ABSTRACT

Wastewaters, mainly the ones from food processing industries, contain considerable amounts of long-chain fatty acids (LCFA). These pollutant compounds, resulting from the hydrolysis of lipids, can be used as energetic resources for the production of biogas. A large amount of methane can be produced from LCFA; theoretically 1g of oleate, one of the most common LCFA found in wastewaters, can be converted to 1.01 L of methane (at standard temperature and pressure), while 1 g of glucose yields only 0.37 L methane. In its core this is a biological process, thus strongly linked to the performance and efficiency of the different microorganisms interacting in the process. Insight into the phylogenetic and functional communities involved in LCFA degradation is necessary to understand and enable the effective performance of bioreactors treating these compounds. In this work we describe the application of culture-dependent and culture-independent strategies to study microbiological and physiological aspects of the degradation of LCFA in anaerobic environments. Two LCFA were used as model substrates: oleate, a mono-unsaturated LCFA (C<sub>18:0</sub>), and palmitate, a saturated LCFA (C<sub>16:0</sub>), both abundant in LCFA-rich wastewaters.

LCFA-degrading communities were developed by selective enrichments growing on oleate and palmitate. Changes in the microbial composition during enrichment were analyzed by DGGE profiling of PCR-amplified 16S rRNA gene fragments. Predominant DGGE-bands of the enrichment cultures were identified by 16S rRNA gene sequencing. A significant part of the retrieved 16S rRNA gene sequences was most similar to those of uncultured bacteria. 16S rRNA gene sequences clustering within the *Syntrophomonadaceae* family were identified as corresponding to predominant DGGE-bands in the oleate- and palmitate-enrichment cultures. In stable palmitate-enrichment cultures members of the *Syntrophobacteraceae* family were also present. Further on, a new obligately syntrophic bacterium, *Syntrophomonas zehnderi*, was isolated from an oleate-degrading culture. This mesophilic, syntrophic, fatty acid oxidizing bacterium degrades straight-chain fatty acids with 4 to 18 carbon atoms but, also, unsaturated LCFA, such as oleate. The presence of *Syntrophomonas zehnderi* related bacteria in several sludges after contact with oleate was, subsequently, verified by DGGE-fingerprinting analysis and suggests its important role in anaerobic oleate degradation in bioreactor sludge. Future work on the performance of bioaugmented reactors with this versatile LCFA-degrading bacterium promise new results on the efficient conversion of LCFA to methane.